

Joint Inflammation Induces Altered Mucin Metabolism and Microbiome Changes in the Ileum and Colon

Meghan M. Moran¹, Jun Li², Quan Shen³, Ankur Naqib^{1,4}, Phillip Engen⁵, Rylan Martin¹, Stefan J. Green⁶, D. R. Sumner¹, Anna Plaas²,

¹Dept of Anatomy & Cell Biology, Rush University Medical Center (RUMC), Chicago, IL, USA; ²Dept Int. Medicine (Rheumatology), RUMC; ³ Dept of Neurosurgery, RUMC; ⁴Bioinformatics Core, RUMC; ⁵Dept Int Medicine, (Gastroenterology) RUMC; ⁶Genomics and Microbiome Core Facility, RUMC

Disclosures: Meghan M. Moran (N), Jun Li (N), Quan Shen (N), Ankur Naqib (N), Philip Engen (N), Rylan Martin (N), Stefan J. Green (N), D. R. Sumner (N), Anna Plaas (N)

Introduction: Inflammatory arthritides, including rheumatoid arthritis (RA), are chronic musculoskeletal diseases that lead to the destruction of cartilage and bone in multiple joints in the body. They lead to painful movements and severely affect quality of life of affected individuals. Recently, impairments of gut health, such as altered microbiota and damaged intestinal barrier function have been reported as additional factors in RA^{1,2}. We have shown that a foreign body reaction to cobalt-chromium particles in the knee joint is accompanied by synovitis, bone loss and an altered gut microbiome composition³. To further understand the mechanisms underlying the gut response to joint inflammation, we used the Complete Freund's Adjuvant (CFA)-induced inflammatory arthritis rat model to examine cell biological reactions and microbiome responses in the ileum and colon.

Methods: The IACUC approved study was carried out with 48 pair-housed male Sprague Dawley rats (375-400g, Envigo). Standard rodent diet and water were provided *ad libitum*. Rats were assigned to one of three study groups: Naïve (no intra-articular injection, IAI, n=10), Sham controls (1x bilateral IAI PBS, n=12), or 1x bilateral IAI CFA (n=26)⁴. M-L knee diameter measurements were collected at Day 0 (pre-IAI) and D 3, 7 and 14 post-IAI. All rats were sacrificed via CO₂ inhalation at D0, 3, 7 or 14 (n = 4-6/group/time). Samples collected at sacrifice include: synovial membranes and distal regions of ileum and colon for mRNASeq; right femur for μ CT⁵; and fecal samples from distal ileum and colon for 16S rRNA gene amplicon-based microbiome characterization³. Ileum and colon were fixed and prepared for "Swiss roll" histology⁶ that included Alcian Blue/Nuclear Red for mucus, biotinylated Lectin histochemistry (GSI, MALII, UEA; Vector Labs) for mucins and anti-CD68 for macrophages. **Statistical Analyses:** One and two-way analyses of variance with Bonferroni corrections were completed using SPSS (v.19, Windows). For 16S Centered Log-Ratio Kruskal Wallis Analysis and for mRNASeq we did differential abundance using edgeR⁷ were completed.

Results: Joint Inflammation: Following IAI CFA, knee swelling developed rapidly from ~9mm at D0 to ~12mm at D3 and remained at ~11.5mm from D7-D14 (p<0.001, 1-way). This was accompanied by increased volume of synovial fluid and synovial pannus formation around the knee joint. Synovial tissue mRNASeq analyses showed significant increases in 40 pro-inflammatory chemokines, interleukins (IL) and their receptors at D3 and 7, with some decreases by D14. mRNA expression for *Pt2rx*, *Cnr1&2*, *Comt*, *P2ry1*, *Scn11a*, *Trpv3*, genes involved in pain transduction, also increased and were highest at D14. Significant trabecular bone loss (p<0.001, 2-way), periosteal hyperplasia, mineralization and adjacent metaphyseal cortical bone pitting were detected. **Gut Microbiome and Cellular Responses:** Microbial relative abundances of several genera were significantly altered in the ileum and colon (Table 1) with the most widespread change at D7. Notably, the relative abundance of bacteria from the genus *Clostridium sensu stricto* (putative mucin degraders), increased at D14 in the ileum and colon. Mucin changes were detected with lectin histochemistry (Fig. 1A). Moreover, increased apical location of tight junction proteins (Claudin2 and Zonulin-1, Fig. 1B,C) was present by D3, indicative of loss of barrier function, but no changes in macrophage distribution or abundance was detected (Fig. 1D). A robust response in gut tissue to joint inflammation was supported by analysis of gut tissue transcriptional patterns (Fig. 2). mRNA abundance of chemokine and IL genes in both ileum and colon were changed, whereas TNF-family genes were altered in the ileum only. Those changes dissipated rapidly in the ileum by D7 but remained altered in the colon. Additionally, alterations were noted in mRNA abundance of genes involved neuropathic pain transduction, in both gut regions and these occurred throughout the post-IAI period. Furthermore, mucin and their specific O-glycosylation genes were modified. Collectively, the data supports the current consensus that gut physiology is modified by induced joint inflammation.

Discussion: The data presented confirm previous reports that injurious stimuli to joint tissue results in concurrent alterations in gut 'health'. To define modifications in the microbiome and cellular responses in gut tissues that could serve as novel therapeutic targets to treat impaired gut function in musculoskeletal diseases, we applied histopathological evaluation along the entire length of ileum and colon and RNAseq analyses of the two tissues. Our data show for the first time that inflammatory joint diseases not only result in defective gut permeability⁸, but that mucin metabolism, an important regulator of gut barrier function and microbial colonization⁹ is also affected. Increase in the relative abundance of putative mucin-degrading Clostridia indicates that joint inflammation modulates mucin metabolism in both ileum and colon. Most importantly, modulation of transcriptional profiles of genes in pro-inflammatory and pain pathways in synovium and gut tissues showed distinctive yet parallel temporal responses.

Significance/Clinical Relevance: The data presented provide new possibilities for mitigating gut involvement in inflammatory joint disease by targeting permeability defects^{10, 11}. Moreover, changes in mucin populations or glycosylation patterns seen in this rat model may provide the basis for developing novel clinically relevant fecal prognostic and diagnostic biomarker approaches to monitor gut health in musculoskeletal diseases.

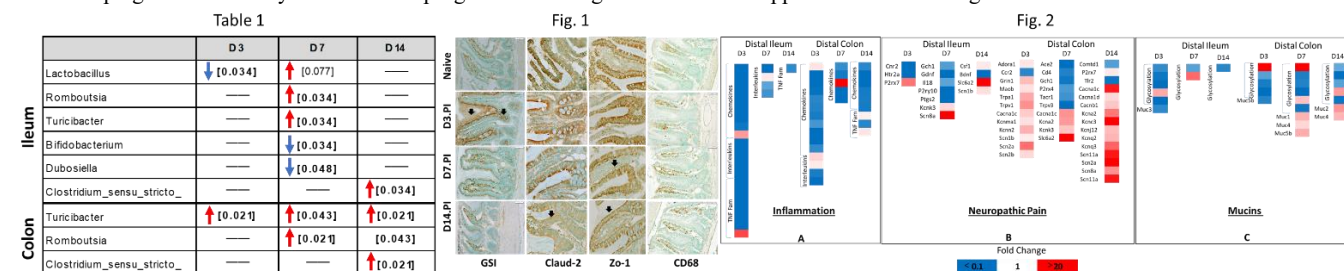


Table 1- Significantly altered taxa (genus level) in ileum and colon vs. D0. **Fig. 2-** Ileum IHC for GSI (A), Claudin-2 (B), Zonulin-1 (C) and CD68 (D) at D3, 7, and 14. **Fig. 3-** Heatmap of host transcriptional profiles (mRNA expression data) of ileum and colon <0.1 to > 20-fold change.

PMID Citations: 1- 32443535; 2- 36864473; 3- 32931052; 4- 21749865; 5- 25689288; 6- 35242614; 7- 19910308; 8- 26331579; 9- 37070751.; 10- 31499514; 11- 29545038